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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

February 14, 2001

MEMORANDUM

Subject: **Methyl Bromide** - Review of Subchronic (4-week) Inhalation Toxicity and Acute/Short-Term Inhalation Toxicity Studies in Dogs

P.C. CODE: 053201 TOX. CHEM. NO.: 555
DP Barcode: D210630 SUBMISSION CODE: S479359
MRID NO.: 443386801 and 443386802

To: Joseph Nevola
Special Review Branch
Special Review and Reregistration Division (7508C)

From: Paul Chin, Ph.D. Paul Chin 2/15/01
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Chemical Manufacturers Association (Methyl Bromide Industry Panel) submitted Subchronic (4-week) Inhalation Toxicity and Acute/Short-Term Inhalation Toxicity Studies in Dogs. These studies have been reviewed and the DERs are attached. The citations and conclusions are presented below:

CITATION: Newton, Paul E. (1994) An Up and Down Acute Inhalation Toxicity Study of Methyl Bromide in the Dog. Pharmaco LSR, Inc. (East Millstone, NJ). Laboratory study number 93-6067 (Sponsor Study No. MBIP-32.0-SDOG-PHARM), September 14, 1994. MRID 43386801. Unpublished study.

EXECUTIVE SUMMARY: In an acute/short-term inhalation toxicity range-finding study (MRID 43386801), methyl bromide (tech., 100% a.i.) was administered to beagle dogs by whole-body inhalation using 2 protocols: Part A ("up-and-down" study)- a total of 6 dogs (3 dogs/sex), 1 dog/exposure group, were exposed in the following order at chamber concentrations of 314, 233, 314, 394, 350 or 345 ppm (equivalent to 1.21, 0.903, 1.52, 0.903, 1.357 or 1.228 mg/L) for one 7-hour exposure. Clinical signs (during/post-exposure) and body weights were evaluated. Part B (repeated-exposure range-finding study for longer-term inhalation studies) - 10 dogs (5 dogs/sex) were exposed for 7 hr/day to methyl bromide as follows: 3 dogs/dose group to 268 or 283 ppm (1.039 or 1.097 mg/L; same animals used in Part A) for up to 2 exposures (terminated early due to toxicity) and 2 new animals/dose group to 55 or 156 ppm (0.213 or 0.604 mg/L) for a total of 4 exposures (the lower 2 exposure levels added after excessive toxicity was observed at higher levels). Clinical signs (during and post-exposure), body weight, hematology, clinical chemistry, organ weights, gross findings and microscopic effects on the brain were evaluated.

Part A: Clinical signs of toxicity were observed during exposure at all levels with dose-dependent severity. At 233 and 314 ppm, neurological symptoms (tremors or trembling extremities, digging, decreased activity, restless behavior), eye blinking, panting and hunched appearance, most after 5-7 hrs exposure, were observed; post-exposure, one 314 ppm animal had increased bronchovesicular sounds 1 day post-exposure and 1 had decreased skin turgor. At ≥345 ppm, similar symptoms, plus respiratory distress and excessive salivation were observed, sometimes by 3-4 hrs' exposure; post-exposure, all showed some clinical signs and increased bronchovesicular sounds. The 345 and 394 ppm exposures were terminated after 5-6 hrs due to excessive respiratory distress. The animal exposed to 394 ppm was also lethargic after exposure. There were no clear effects on body weight. The study author selected 325 ppm as the maximum tolerated concentration and 270 ppm as a lower exposure level for Part B.

Part B: At 156 ppm, decreased activity, prostration, labored breathing (3rd exposure), lacrimation (1st exposure), irregular gait (post-exposure) and decreased body weights (8%-10% below pretest) were observed. At 268 ppm, decreased activity, labored breathing (by 2nd exposure), irregular gait, recumbency, white gums, excessive salivation (post-exposure) and delirium, thrashing, pulmonary edema/rales, vocalization and tremors (detailed post-exposure clinical exam) and fluid in lungs (2 females) were reported. At 283 ppm during exposures, decreased activity, labored breathing including rales, excessive salivation and emesis were observed and post-exposure, marked ataxia, emesis, excessive salivation, signs of dehydration, pale mucous membranes, depression and ocular irritation (dry cornea, scleral infection) were observed (all animals showed several or all signs). Animals were sacrificed before or after the 2nd exposure and all showed weight loss on day 2 (8%-13% below pretest). One male had fluid and red discoloration in the lungs. No effects were observed at 55 ppm and no treatment-related effects were observed on hematology, clinical chemistry, organ weights or brain pathology at any dose. **The systemic toxicity LOAEL (4 days' exposure) is 156 ppm (0.604 mg/L), based on clinical signs and decreased body weight. The NOAEL is 55 ppm (0.213 mg/L).**

This acute/short-term inhalation toxicity study is classified **Acceptable/Nonguideline**. The

study was not required by the Agency, but was conducted for the California Department of Pesticide Regulation as a range-finding study for dog subchronic/chronic inhalation toxicity studies on methyl bromide.

CITATION: Newton, Paul E. (1994) A Four Week Inhalation Toxicity Study of Methyl Bromide in the Dog. Pharmaco LSR, Inc. Toxicology Services North America (East Millstone, NJ). Laboratory Study No. 93-6068 (Sponsor Study No. MBIP-33.0-DOGRF-PHARM), September 14, 1994. MRID 43386802. Unpublished.

EXECUTIVE SUMMARY: In a subchronic inhalation study (MRID 43386802) methyl bromide (tech., 100% a.i.) was administered 7 hours/day, 5 days/week to 4 beagle dogs/sex/dose by whole body exposure at target concentrations of 0, 5, 10/150, 25, 50 or 100 ppm (actual mean concentrations 0, 5.3, 11.0/158.0, 26.0, 53.1 or 102.7 ppm; equivalent to 0, 0.021, 0.043/0.614, 0.101, 0.206 or 0.399 mg/L), as follows: "interim" sacrifice - 2 dogs/sex, 0 ppm group and all dogs, 25, 50 and 100 ppm groups, for almost 5 weeks (total 24 exposures); "terminal sacrifice" - 2 dogs/sex, 0 ppm group and all dogs, 5 ppm group for almost 7 weeks (total 34 exposures); and all dogs, 10/150 ppm group for almost 5 weeks at 10 ppm (24 exposures), then at 150 ppm for 6 additional exposures (also "terminal" sacrifice). In addition to standard evaluations performed in a guideline subchronic study, a neurological examination was performed by a veterinarian after termination of exposures and serum bromide levels were measured weekly.

"Interim" sacrifice: At 53 ppm, decreased activity was observed in 2/8 dogs during exposure beginning on day 14. At 103 ppm, decreased activity (starting on day 9 in 3 dogs; eventually in all), tremors (1 male, 10th exposure) and decreased body weight in both sexes (-9% less than controls due to cumulative weight loss of -0.6 kg, males and -1.0 kg, females) were observed. Serum bromide ion concentration was increased for both sexes at 53 and 103 ppm (+160% to +500%), with only slight increases at 26 ppm (by day 19, +44%, males and +37%, females). No neurological deficits were observed during the post-exposure neurological examination. There were no treatment-related effects on food consumption, ophthalmological findings, hematology parameters, organ weights or gross findings at any dose, and no effects observed at ≤26 ppm. **The systemic toxicity LOAEL for 5 weeks (24 exposures) is 53 ppm (0.206 mg/L), based on decreased activity. The NOAEL is 26 ppm (0.101 mg/L).**

"Terminal" sacrifice: At 5 ppm after 34 exposures, 2/8 dogs (both females) showed unresponsiveness and/or depressed appearance at the neurological exam. At 158 ppm (increased from 10 ppm after the 24th exposure), 3 males were sacrificed after 6 exposures due to opisthotonos, paddling gait of all limbs, opening/closing of jaws and convulsions. Decreased activity (after 2 exposures); poor condition and in 1 male, tremors and prostration (6 exposures); at 2 days after the last exposure, ataxia, intention tremor, nystagmus, marked depression and inability to perform postural responses; decreased body weight/weight loss (males -24%/loss of 1.2 kg; females loss of 0.7 kg); increased urinary bilirubin and protein; vacuolization of the cerebellar granular layer (8/8); olfactory epithelial degeneration in the nasoturbinal tissues (8/8) and intracytoplasmic vacuolization of the adrenal gland *zona fasciculata* (4/4 males) were

observed. Serum bromide levels increased by up to ~400% in both sexes. No treatment-related findings were reported in animals exposed to 11 ppm for 5 weeks. There were no treatment-related effects on food consumption, ophthalmological findings, hematology parameters, organ weights or gross findings in this study at any dose. **The systemic LOAEL (threshold) for a 7 weeks (34 exposures) is 5 ppm (0.021 mg/L), based on decreased responsiveness in females. The NOAEL (threshold) is <5 ppm.**

This subchronic toxicity study is classified **Acceptable/Non-Guideline** (§82-4). A subchronic inhalation study in the dog was not required by the US EPA for reregistration of methyl bromide; this study was conducted as a range-finding study for a chronic inhalation study in dogs to satisfy data requirements of California Department of Pesticide Regulation.

C:\1-new-file\methylbromide\ac.subchr in dogs 2/14/2001

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[METHYL BROMIDE, TECH.] **Acute/Short-Term Inhalation Study (Range-Finding)**

EPA Reviewer: Linnea Hansen, Ph.D.
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Byong-Han Chin, Ph.D.
Toxicology Branch I (7509C)

Date 1/29/01

Date 1/31/01

DATA EVALUATION RECORD

STUDY TYPE: "Up and Down" Acute/Short-Term Inhalation Toxicity - ~~Rat~~ Dog; Non-guideline (range-finding study submitted under §81-3)

DP BARCODE: D210630
P.C. CODE: 053201

SUBMISSION CODE: S479359
TOX. CHEM. NO.: 555

TEST MATERIAL (PURITY): Methyl bromide (tech., 100% a.i.)

SYNONYMS: Bromomethane

CITATION: Newton, Paul E. (1994) An Up and Down Acute Inhalation Toxicity Study of Methyl Bromide in the Dog. Pharmaco LSR, Inc. (East Millstone, NJ). Laboratory study number 93-6067 (Sponsor Study No. MBIP-32.0-SDOG-PHARM), September 14, 1994. MRID 43386801. Unpublished study.

SPONSOR: Chemical Manufacturers Association (Methyl Bromide Industry Panel), Washington, D.C.

EXECUTIVE SUMMARY: In an acute/short-term inhalation toxicity range-finding study (MRID 43386801), methyl bromide (tech., 100% a.i.) was administered to beagle dogs by whole-body inhalation using 2 protocols: Part A ("up-and-down" study)- a total of 6 dogs (3 dogs/sex), 1 dog/exposure group, were exposed in the following order at chamber concentrations of 314, 233, 314, 394, 350 or 345 ppm (equivalent to 1.21, 0.903, 1.52, 0.903, 1.357 or 1.228 mg/L) for one 7-hour exposure. Clinical signs (during/post-exposure) and body weights were evaluated. Part B (repeated-exposure range-finding study for longer-term inhalation studies) - 10 dogs (5 dogs/sex) were exposed for 7 hr/day to methyl bromide as follows: 3 dogs/dose group to 268 or 283 ppm (1.039 or 1.097 mg/L; same animals used in Part A) for up to 2 exposures (terminated early due to toxicity) and 2 new animals/dose group to 55 or 156 ppm (0.213 or 0.604 mg/L) for a total of 4 exposures (the lower 2 exposure levels added after excessive toxicity was observed at higher levels). Clinical signs (during and post-exposure), body weight, hematology, clinical chemistry, organ weights, gross findings and microscopic effects on the brain were evaluated.

Part A: Clinical signs of toxicity were observed during exposure at all levels with dose-dependent severity. At 233 and 314 ppm, neurological symptoms (tremors or trembling extremities, digging, decreased activity, restless behavior), eye blinking, panting and hunched appearance, most after 5-7 hrs exposure, were observed; post-exposure, one 314 ppm animal had

increased bronchovesicular sounds 1 day post-exposure and 1 had decreased skin turgor. At ≥345 ppm, similar symptoms, plus respiratory distress and excessive salivation were observed, sometimes by 3-4 hrs' exposure; post-exposure, all showed some clinical signs and increased bronchovesicular sounds. The 345 and 394 ppm exposures were terminated after 5-6 hrs due to excessive respiratory distress. The animal exposed to 394 ppm was also lethargic after exposure. There were no clear effects on body weight. The study author selected 325 ppm as the maximum tolerated concentration and 270 ppm as a lower exposure level for Part B.

Part B: At 156 ppm, decreased activity, prostration, labored breathing (3rd exposure), lacrimation (1st exposure), irregular gait (post-exposure) and decreased body weights (8%-10% below pretest) were observed. At 268 ppm, decreased activity, labored breathing (by 2nd exposure), irregular gait, recumbency, white gums, excessive salivation (post-exposure) and delirium, thrashing, pulmonary edema/rales, vocalization and tremors (detailed post-exposure clinical exam) and fluid in lungs (2 females) were reported. At 283 ppm during exposures, decreased activity, labored breathing including rales, excessive salivation and emesis were observed and post-exposure, marked ataxia, emesis, excessive salivation, signs of dehydration, pale mucous membranes, depression and ocular irritation (dry cornea, scleral infection) were observed (all animals showed several or all signs). Animals were sacrificed before or after the 2nd exposure and all showed weight loss on day 2 (8%-13% below pretest). One male had fluid and red discoloration in the lungs. No effects were observed at 55 ppm and no treatment-related effects were observed on hematology, clinical chemistry, organ weights or brain pathology at any dose. **The systemic toxicity LOAEL (4 days' exposure) is 156 ppm (0.604 mg/L), based on clinical signs and decreased body weight. The NOAEL is 55 ppm (0.213 mg/L).**

This acute/short-term inhalation toxicity study is classified **Acceptable/Nonguideline**. The study was not required by the Agency, but was conducted for the California Department of Pesticide Regulation as a range-finding study for dog subchronic/chronic inhalation toxicity studies on methyl bromide.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Methyl bromide
   Description: technical grade colorless gas
   Lot/Batch #: SLV (Great Lakes Chemical Corp.)
   Purity: 100% a.i.
   Stability of compound: not indicated; however, methyl bromide is stable stored pressurized at room temperature.

CAS #: 74-83-9

2. Vehicle and/or positive control: none

3. Test animals:
Species: dog
Strain: beagle
Age and weight at study initiation: 7 months
Source: Marshall Farms, U.S.A., Inc., North Rose, NY
Housing: Individually housed in elevated metal grid cages (except during exposures)
Diet: PMI Feeds, Inc. Certified Canine Diet® No. 5007. Four hundred grams were provided for 2 hr/day (except during exposures).
Water: tap water *ad libitum* (except during exposures).
Environmental conditions: Temperature: 20° to 26°C
Humidity: 38% to 56%
Air changes: not indicated
Photoperiod: 12 hr light/12 hr dark
Acclimation period: For Group A (single exposures), 2 weeks; for Group B (repeated exposure), 1 week.

B. STUDY DESIGN:

1. In life dates - start: February 14, 1994; end: March 27, 1994

2. Purpose of study

This study was conducted as range-finder to estimate maximum tolerated exposures in beagles for longer-term studies. Part A, an up-and-down acute exposure study based on the protocols of Dixon and Massay[1] and of Bruce[2], was conducted first to determine the maximum tolerated dose for a 7-hour exposure (1 animal/dose group). Following initial exposure at 314 ppm, 5 more animals were tested at higher or lower concentrations to determine the MDT, depending on the clinical signs of toxicity observed. Based on those results, 2 dose levels were selected for Part B. Part B (3 animals/group) was conducted to determine the maximum tolerated exposure level for four consecutive daily exposures of 7 hours' duration. Two groups were added for exposure at 50 (55 ppm) and 100 ppm (156 ppm) due to excessive toxicity at 268 and 283 ppm. Dose levels tested in each study part are shown below in Tables 1 and 2.

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[1] *Introduction to Statistical Analysis*, 3rd Ed. (1969), McGraw Hill, NY

[2] *Fundam. Appl. Tox.* 5:151-197 (1985)

3. Animal assignment

Animals were assigned by an unspecified method to the test groups (Part A, Table 1 and Part B, Table 2. In Part A, exposure levels were adjusted up and down in the order shown to determine a maximum tolerated exposure level (1 animal/group).

TABLE 1: STUDY DESIGN FOR PART A, "UP AND DOWN" ACUTE EXPOSURES [a]

Test group	Nominal Conc. (ppm)	Analytical Conc. (ppm)	Exposure Duration (hrs)	MMAD μm	Animal no./sex
1	300	**314±19**	7	nm[b]	13140/♀
2	230	**233±21**	7	nm	13120/♂
3	300	**314±6**	7	nm	13122/♂
4	390	**394±20**	7	nm	13145/♀
5	345	**350±13**	7	nm	13134/♂
6	345	**345±8**	7	nm	13152/♀

a Data extracted from MRID 43386801, table on p. 14.
b Not measurable (no detectable aerosol).

In Part B, 2 or 3 animals/group were exposed as shown below in Table 2. Animals in the 268 and 283 ppm groups were the same animals used in Part A. Because of the excessive toxicity at 268 and 283, exposures were discontinued after 2 days. The 50 and 100 ppm dose groups were added when excessive toxicity was observed at 268 and 283 ppm.

TABLE 2: STUDY DESIGN FOR PART B, 4-DAY EXPOSURES [a]

Test group	Nominal Conc.(ppm)	Analytical Conc. (ppm)	Exposure Duration (days)	MMAD μm	Animal no./sex
1	270	**268±19**	4 (7h/day)	nm[b]	13120/♂ 13140/♀ 13145/♀
2	325	**283±13**	4 (7h/day)	nm	13122/♂ 13134/♂ 13152/♀
3[c]	50	**55±8**	4 (7h/day)	nm	13162/♂ 13175/♀
4[c]	100	**156±15**	4 (7h/day)	nm	13164/♂ 13179/♀

a Data extracted from MRID 43386801, table on p. 15.
b nm = Not measurable (no aerosol detected).
c These groups were added after excessive toxicity was observed in the original dose groups, 1 and 2.

4. Generation of the test atmosphere and description of the chamber: In Part A, dogs were exposed individually in 1,000 liter glass and stainless-steel exposure chambers (Wahmann, Timonium, MD). Chambers were operated dynamically under a slightly negative pressure at an airflow rate of 215 liters/min. Complete air changes were accomplished every 4.7 min. and the time to equilibrium (T_{99}) was 20 min. In Part B, groups of 2 to 3 dogs were exposed in 6,000 liter chambers (Harford, Aberdeen, MD) which were operated dynamically under slight negative pressure. An airflow rate between 1,243 to 1,291 liters per minute provided complete air changes every 4.6 - 4.8 minutes and a T_{99} of 21 - 22 minutes. In both study parts, temperature was maintained between 20-23°C and humidity between 33 to 66% (measurements were taken preexposure and about every 30 minutes during exposures). Airflow rates for each chamber were reported to keep the animal loading factor below 5% and oxygen concentration above 19%. Atmospheres were generated by mixing methyl bromide from the compressed gas cylinders in a gas bag before being metered into the exposure chambers. Animals remained in the chambers for 30 minutes after termination of exposure to allow clearance of the test atmosphere. Nominal concentration in ppm was determined as follows:

$$\frac{\text{total volume of test material used during exposure}}{\text{total air volume passing through chamber during exposure}}$$

Test atmosphere concentration: Chamber concentrations of methyl bromide were measured using a MIRAN® Ambient Air analyzer with a strip chart recorder and digital multimeter at a wavelength of 7.5 μm and pathlength of 11.83 meters. Chamber atmosphere was drawn constantly through the analyzer and measurements recorded from the main sampling portal at least hourly and from the distribution sampling portal once per exposure. Results are summarized in Tables 1 and 2, above.

Particle size determination was measured hourly using a TSI Aerodynamic Particle Sizer with a diluter. There were no measurable aerosol particles during the exposures.

5. Statistics - Statistics were not performed because of the small number of animals used.

C. METHODS:

1. Observations:

For Parts A and B of this study, animals were inspected at least twice daily for signs of toxicity and mortality. During exposure periods, animals were observed every 15 minutes during the first hour and hourly for the remaining exposure time. A detailed physical examination was conducted pretest and daily following the exposures.

2. Body weight

In Part A, animals were weighed pretest and daily between days 1 and 14. In Part B, animals were weighed pretest, daily on days 1 to 4, and immediately before necropsy.

3. Food consumption

Food consumption for each animal was not determined because the test material was administered by inhalation.

4. For Part B animals only, blood was collected from fasted animals via the jugular vein for hematology and clinical analysis pretest and prior to sacrifice. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	*Blood clotting measurements**		
X	(Prothrombin time)		
X	(Partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein Electrophoresis
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

5. Sacrifice and Pathology

For Part B animals only, all animals that died or were sacrificed on schedule were subjected to gross pathological examination. However, only adrenal glands, brain, kidneys, liver, lungs with mainstem bronchi and testes with epididymides were preserved (lungs were infused with formalin to ensure fixation). Only brain (3 sections at medulla/pons, cerebellum and cerebrum) was examined microscopically. Adrenal glands, brain, kidneys, liver, lungs and testes/epididymides were weighed.

II. RESULTS - STUDY PART A

A. Observations

1. Toxicity - Signs of toxicity were observed in a dose-dependent manner in all animals. Results are summarized below in Table 3 (in-chamber observations), Table 4 (post-exposure cageside observations) and Table 5 (post-exposure clinical examinations):

TABLE 3: OBSERVATIONS DURING EXPOSURE, PART A[1]

	EXPOSURE IN PPM					
Observation	**233**	**314**	**314**	**345[2]**	**350**	**394[3]**
Trembling extremities	P (5)[4]	P (7)	--[5]	--	--	--
Panting	P (6)	--	--	--	--	--
Rapid blinking of eyes	P (6-7)	--	--	--	--	--
Tremors	P (7)	--	P (5-7)	P (3-7)	P (5-7)	P (3-5)
Hunched appearance	--	P (7)	P (6-7)	P (5-6)	P (5-7)	P (3-5)
Digging	--	P (7)	--	--	--	--
Decreased activity	--	--	P (4)	--	P (4-7)	--
Restless behavior	–	–	P (6)	--	--	--
Excessive salivation	--	--	--	P (6)	P (6-7)	--
Labored breathing	--	--	--	P (5-6)	P (3-7)	--
Gasping	--	--	--	P (5-6)	--	--
Swallowing response	--	--	--	--	P (6-7)	--

1 *Data extracted from narrative part of Results Section and Table 3 of MRID 43386801.*
2 Exposure terminated at the 7th hr due to mucoid nasal discharge and salivation.
3 Exposure terminated at the 6th hr due to mucoid nasal discharge and labored breathing.
4 P = present (numbers in parentheses indicate hour of exposure when observed).
5 -- Not observed

TABLE 4: POST-EXPOSURE SIGNS OF TOXICITY, CAGESIDE AND CLINICAL VETERINARIAN EXAMINATION[1]

Observation	Exposure in ppm					
	233	**314**	**314**	**345**	**350**	**394**
No observed abnormalities	P (1-14)[2]	P (1-14)	P (1-14)	P (2-14)	P (2-14)	P (2-14)
White nasal discharge	--	--	--	M[3] (1)	M (1)	E[4] (1)
Lethargic	--	--	--	--	--	P (1)
Excessive salivation	--	--	--	P (1)	P (1)	P (1)
Panting	--	--	--	P (1)	P (1)	P (1)

1 Data extracted from Table 3, MRID 43386801. Data not analyzed statistically.
2 P = present. Numbers indicate the days of the study, between day 1 and day 14 post-exposure, when observations were reported.
3 M = moderate
4 E = excessive
– Not observed

TABLE 5: CLINICAL VETERINARIAN EVALUATIONS, POST-EXPOSURE[1]

Exposure, ppm	Study day	Animal No.	Findings
314	2	13140	Increased bronchovesicular sounds, both hemithoraces.
314	2	13122	Decreased skin turgor (mucous membranes and corneas within normal limits).
345	2	13152	Slightly increased bronchovesicular sounds, both hemithoraces.
350	1	13134	Increased bronchovesicular sounds, both hemithoraces; excessive salivation; respiratory stridor; slightly decreased activity.
394	1 2	13145	Rales, dull, depressed, lethargic. Slightly increased bronchovesicular sounds, both hemothoraces.

1 Data extracted from Table 3 of MRID 43386801.

No symptoms were observed before 3 hrs of exposure or by day 2 post-exposure at any dose level. The initial exposure, 314 ppm, caused hunched appearance, trembling of extremities and digging during the last hour of exposure. No findings were observed immediately post-exposure, but bronchovesicular sounds (indicating pulmonary edema) were observed on the following day during the clinical examination.

The second exposure, 233 ppm, caused trembling extremities, tremors, panting and rapid blinking by 5-7 hrs of exposure, but no post-exposure effects were observed.

During the third exposure, also at 314 ppm, hunched appearance and tremors (by 5-7 hrs) and decreased activity (4th hr), followed by restless behavior (6th hr), were observed. Because there were no post-exposure findings (except for decreased skin turgor at the veterinary examination), the fourth exposure was conducted at 394 ppm.

At 394 ppm, tremors and hunched appearance were observed by the 3rd hr of exposure and exposure was terminated at 6 hrs because of excessive respiratory distress (labored breathing, mucoid nasal discharge). Clinical signs persisted post-exposure (lethargy, nasal discharge, panting, salivation) and rales or bronchovesicular sounds were observed post-exposure and on the day after exposure.

The fifth exposure was conducted at 350 ppm and also resulted in respiratory distress, with labored breathing occurring by 3 hrs exposure and tremors, hunched appearance, decreased activity, excessive salivation and swallowing response observed between 4-7 hrs. Post-exposure, nasal discharge, excessive salivation and panting were reported. Increased bronchovesicular sounds and rales were also observed during the post-exposure clinical examination.

The final exposure at 345 ppm resulted in similar symptoms and onset, during and post-exposure, to those seen at 350 ppm, with slightly later onset of respiratory difficulty during exposure (5 hrs vs. 3 hrs). Because mucoid nasal discharge and salivation were observed, exposure was terminated at the 7th hour. Bronchovesicular sounds were reported on the day after exposure.

Based on the severity of clinical symptoms and persistence post-exposure, particularly signs of pulmonary edema, a maximum tolerated exposure level of 325 ppm was selected as the target high dose in Part B of this study (target low dose of 270 ppm selected).

2. Mortality - There was no mortality resulting from exposures to methyl bromide.

B. Body weight and weight gain

No treatment-related effects on body weight were reported.

III. RESULTS - STUDY PART B

A. Observations

1. Toxicity - Clinical signs of toxicity observed during exposures are shown below in Table 6. Signs observed cageside post-exposure are shown below in Table 7 and findings from the detailed clinical exam in Table 8:

TABLE 6: CAGESIDE CLINICAL OBSERVATIONS DURING EXPOSURE[1]

		EXPOSURE IN PPM			
Observation/exposure day		**55 (N = 2)**	**156 (N = 2)**	**268 (N = 3)**	**283 (N = 3)[2]**
Lacrimation	1	0	1 (5-7)[3]	0	0
	2	0	0	0	0
	3	0	2 (2-7; 4-7)	--[4]	--
	4	0	2 (1-7; 3-7)	--	--
Labored breathing	1	0	0	0	3 (6; 6-7; 6-7)
	2	0	0	1 (3-7)	2 (0.5-5)[5]
	3	0	2 (3-7; 4-7)	--	--
	4	0	2 (5-7)	--	--
Prostration	1	0	0	0	0
	2	0	0	0	0
	3	0	1 (5-7)	--	--
	4	0	0	--	--
Decreased activity	1	0	0	0	0
	2	0	0	3 (7)	2 (0.25-5)
	3	0	2 (2-7)	--	--
	4	0	2 (3-7)	--	--
Excessive salivation	1	0	0	0	2 (6-7; 7)
	2	0	0	0	2 (5)
	3	0	0	--	--
	4	0	0	--	--
Emesis	1	0	0	0	2 (6-7; 7)
	2	0	0	0	2 (3-4; 3-5)
	3	0	0	--	--
	4	0	0	--	--

1 Data extracted from Table 3-5, MRID 43386801.
2 Animal 13152 was sacrificed prior to day 2 exposure because of severe toxicity.
3 Number of animals affected (values in parentheses indicate hour of exposure during which effects were seen).
4 -- Not observed on these days; exposures discontinued after day 2 because of excessive toxicity.
5 Day 2 exposures at 283 ppm were terminated after 5 hrs due to severe toxicity.

TABLE 7: CAGESIDE CLINICAL OBSERVATIONS POST-EXPOSURE[1]

	Exposure in ppm			
	55	**156**	**268[2]**	**283[3]**
Irregular gait	--[4]	2 (4)[5]	1 (2)	--
Recumbency	--	--	1 (2)	--
White gums	--	--	3 (2)	1 (1)
Excessive salivation	--	--	1 (2)	2 (1)
Decreased activity	--	--	--	1 (1)
White nasal discharge	--	--	--	1 (1)
White oral discharge	--	--	--	1 (1)
Labored breathing	--	--	--	1 (1)

1 Data extracted from Table 3-6, MRID 43386801.
2 Exposures not continued after day 2.
3 Exposures not continued after day 1.
4 -- not observed.
5 Number of animals affected (day or days on which symptoms observed).

In addition to the above cageside observations, animals exposed to 268 or 283 ppm showed symptoms during the detailed examination by a veterinarian. These findings are summarized for each animal in Table 8, below:

TABLE 8: CLINICAL FINDINGS, DETAILED POST-EXPOSURE VETERINARIAN EXAMINATION[1]

Exposure, ppm	Study day	Animal no.	Findings
268	2	13120	Severe delirium, acute onset Thrashing and traumatizing behavior Vocalization
	2	13140	Increased respiratory effort Mild tremors Pulmonary edema/rales Slightly cyanotic mucous membranes
	2	13145	Extreme delirium, acute onset Thrashing Vocalization Pulmonary edema/rales
283	2	13122	Emesis Depression Pale mucous membranes Excessive salivation Marked ataxia Increased bronchvasicular sounds/marked expiratory grunt and effort/rales Cachectic appearance (dehydration?)
	2	13134	Depressed/depressed mentation Trembling Emesis Scleral infection, bilateral Slightly pale mucous membranes Excessive salivation Expiratory grunt/increased expiratory effort/rales Marked ataxia Decreased skin turgor, cachectic appearance (dehydration?)
	2	13152	Dull/depressed Recumbent Pink and tacky mucous membranes Decreased skin turgor Dry corneas, bilateral Marked ataxia

1 Data extracted from Table 3-7, MRID 43386801.

No symptoms were reported during or after exposure at 55 ppm. At ≥156 ppm, clinical signs observed during and post-exposure demonstrated irritation of the respiratory tract and neurotoxicity, particularly on gait, motor control and level of activity. Symptoms were observed at all exposure levels. At 156 ppm during exposures, lacrimation, labored breathing, decreased activity (and in 1 animal, prostration) were reported. Signs were generally first observed by Day 3, except for lacrimation, first observed during the last 2 hrs of Day 1. Post-exposure, 2 animals showed irregular gait after the 4th exposure.

At 268 ppm during exposures, labored breathing and decreased activity were observed during the 2nd exposure. However, post-exposure, more pronounced respiratory effects (including pulmonary edema/rales, difficulty breathing) and neurobehavioral symptoms (tremors, thrashing, tremors, delirium and vocalization) were observed. No additional exposures were conducted after the 2nd day. At 283 ppm during exposures, labored breathing was observed towards the end of the first exposure and as early as within the first hour of exposure on the 2nd exposure day. Also observed were decreased activity on day 2 and excessive salivation and emesis on days 1 and 2. Post-exposure, these findings continued on study day 1 and 2. Marked ataxia, depression, decreased skin turgor or pale/tacky mucous membranes and rales were also noted. Because of the toxicity observed, exposures were terminated after the first or during the second exposure (see also Mortality, below).

2. Mortality - Although no animals died during the study, the following animals were humanely sacrificed due to excessive toxicity: one female (13152) exposed to 283 ppm was sacrificed prior to the 2nd exposure and the remaining animals (males) at this exposure level during the 2nd exposure (at 5 hrs); at 268 ppm, all animals were sacrificed following the 2nd exposure.

B. Body weight and weight gain - Individual body weights are shown below in Table 9:

TABLE 9: INDIVIDUAL ANIMAL BODY WEIGHTS, STUDY PART B[1]

Exposure level, ppm	Animal No.	Study day				
		Pretest	1	2	3	4
55	13162	7.9	7.4	7.7	7.8	7.7
	13175	9.7	9.5	9.8	9.9	9.8
156	13164	10.4	10.8	10.7	9.9	9.6
	13179	8.4	8.1	7.9	8.2	7.6
268	13120	9.5	9.4	9.1	--[2]	--
	13140	8.3	8.0	8.6	--	--
	13145	8.9	8.6	8.8	--	--
283	13122	8.3	7.9	7.6	--[2]	--
	13134	8.0	7.5	7.3	--	--
	13152	6.9	6.6	6.0[3]	--	--

1 Data extracted from Table 4-2, MRID 43386801. Not analyzed statistically.
2 -- Not determined. Animals in these groups were sacrificed due to severe toxicity.
3 Sacrificed prior to second exposure due to excessive toxicity.

There were no effects on body weight among dogs exposed to methyl bromide for 4 days at 55 ppm. At 156 ppm, decreases of -8% to -10% of pretest weights were observed by day 4. There were no significant changes in body weight in dogs exposed to 268 ppm for 2 days. However, at 283 ppm weight losses ranging from -8% to -13% of pretest weights were observed on day 2. These decreases appeared to be treatment-related.

C. Blood work

1. Hematology - Selected individual animal hematology findings are shown below in Table 10:

TABLE 10: SELECTED INDIVIDUAL ANIMAL HEMATOLOGY VALUES[1]

Exposure in ppm/ Animal no.	HGB (g/dl)	HCT (%)	RBC (10^6/ml)	WBC (10^3/ml)
Pretest				
55 13162	15.2	45.7	6.84	6.92
13175	17.5	52.6	7.55	8.09
Mean	16.4	49.2	7.20	7.50
156 13165	17.4	53.1	7.75	8.48
13179	15.5	46.7	6.84	8.74
Mean	16.4	49.9	7.30	8.61
268 13120	16.9	50.6	7.52	6.87
13140	16.9	50.1	7.21	6.46
13145	15.5	46.8	6.63	9.27
Mean	16.4	49.2	7.12	7.53
283 13122	15.2	45.6	6.94	16.80
13134	14.7	44.1	6.43	18.54
13152	15.6	46.8	6.91	6.71
Mean	15.2	45.5	6.76	14.02
Termination				
55 13162	13.8	41.3	6.12	6.52
13175	15.9	48.1	6.84	8.86
Mean	14.8	44.7	6.48	7.69
156 13164	18.8	57.2	8.25	11.50
13179	15.7	48.2	6.94	9.36
Mean	17.2	52.7	7.60	10.43
268 13120	20.2	60.4	8.78	18.03
13140	19.8	58.8	8.50	16.69
13145	17.6	54.0	7.44	24.26
Mean	19.2	57.7	8.24	19.66
283 13122	21.4	64.8	9.68	6.36
13134	17.1	51.9	7.49	16.01
13152	16.3	49.8	7.30	16.13
Mean	18.3	55.5	8.16	12.83

1 Data extracted from Tables 5-2 and 5-3, MRID 43386801. Not analyzed statistically.

The study authors noted the increases in the parameters shown above, but did not consider

them to be treatment-related effects because they were within historical control range. At 156 ppm and higher, mean hemoglobin, hematocrit, red blood cells and white blood cells were increased relative to pre-exposure values. Because these increases occurred over such a short exposure period (2 days), they may have been stress-related; however the biological significance of these findings is unclear, and a dose-response was not observed.

2. Clinical chemistry - Selected individual animal chemistry values are shown below in Table 11:

TABLE 11: SELECTED INDIVIDUAL ANIMAL CLINICAL CHEMISTRY VALUES[1]

Exposure in ppm/ Animal no.	BUN (mg/dl)	AST (IU/L)	K+ (mg/dl)
Pretest			
55 13162	16.8	36	4.9
13175	11.3	27	4.4
Mean	14.0	32	4.6
156 13165	11.1	32	4.7
13179	17.7	29	4.5
Mean	14.4	30	4.6
268 13120	12.8	21	4.4
13140	13.1	20	4.5
13145	12.7	26	4.4
Mean	12.9	22	4.4
283 13122	16.6	28	4.5
13134	15.0	30	4.5
13152	20.8	47	5.6
Mean	17.5	35	4.9
Termination			
55 13162	19.2	26	4.6
13175	14.2	28	3.9
Mean	16.8	27	4.2
156 13164	12.3	21	4.9
13179	18.8	28	5.6
Mean	15.6	24	5.2
268 13120	25.9	130	3.6
13140	25.7	27	3.7
13145	26.6	60	3.8
Mean	26.1	72	3.7
283 13122	37.6	86	3.1
13134	26.9	31	3.3
13152	40.3	48	3.7
Mean	34.9	55	3.4

1 Data extracted from Tables 7-2 to 7-5, MRID 43386801. Data not analyzed statistically.

Clinical chemistry values of animals exposed to 55 ppm did not show significant

differences from the pretest values. At 268 and 283 ppm, BUN, AST were increased and potassium decreased relative to both the pretest values and to the 55 ppm animals. These values were also increased relative to the historical control data provided by the study authors (267 animals; data source not indicated in report). The historical control values provided are as follows: BUN range 5.9-29 (mean 9.4-18.4); AST range 10-60 (mean 16-28) and potassium range 3.7-7.3 (mean 4.4-6.0). TB-I agreed with the study authors that these increases were treatment-related because they exceeded the available historical control data and were increased relative to both 55 ppm animals and the pretest values. Although ALP and ALT also showed increases at the higher exposure levels relative to the 55 ppm animals (data not shown), TB-I agreed with the study authors that they were probably not treatment related because they did not exceed the available historical control values and were not biologically significantly different from the pretest values.

D. Sacrifice and pathology

1. Organ weight - Organ weights were measured only for the animals that survived to the scheduled necropsy date after 4 days of exposure. The 268 and 283 ppm groups were therefore not determined. Absolute and relative organ weights of the 156 ppm animals did not show significant differences from the 55 ppm animals.

2. Gross pathology - At 283 ppm, one male had fluid and red discoloration in the lungs. At 268 ppm, two females had fluid in the lungs and one had fluid in the trachea. TB-I agreed with the study authors that these findings were treatment-related. No other treatment-related findings were reported in any exposure group.

3. Microscopic pathology - No abnormal microscopic lesions were reported in the brain at any dose tested. No other tissues or organs were examined.

III. DISCUSSION

A. This was a non-guideline study, conducted as a range-finding study to estimate maximum tolerated dose levels for longer-term exposures to beagle dogs. Inhalation toxicity studies in the dog were not required by the Agency, but were required by the State of California Department of Pesticide Regulation. This range-finding study utilized an "up-and-down" single exposure protocol designed to estimate maximum tolerated 7-hr exposure levels, followed by a 4-day range-finding study.

Part A of this study tested single exposure levels of methyl bromide in an "up and down" format, ranging between 233 to 394 ppm. Clinical signs of toxicity were observed in a dose-dependent manner at all exposure levels. The major target organs of methyl bromide following acute exposure were the lungs, as evidenced by respiratory distress, rales and nasal discharge, and the nervous system, with decreased activity/depression,

tremors or trembling limbs and unusual behaviors observed. Excessive salivation was also observed. Based on the findings from Part A, the study author selected 325 ppm as the MTD for a 7 hr exposure to methyl bromide. This was selected as the higher exposure target concentration, with a lower target exposure level of 270 ppm, for testing in Part B.

In Part B of this study, animals were exposed to methyl bromide for 7 hr/day up to 4 consecutive days. Actual concentrations in the initial set of exposures were 268 ppm and 283 ppm, but exposures were terminated after 2 days due to excessive toxicity. Signs observed were similar to those observed following acute exposures, but in addition, at 268 and 283 ppm ataxia or gait irregularities, and at 283 ppm, emesis and signs consistent with dehydration and ocular irritation were reported. In addition, weight loss was observed at 283 ppm and at both 268 and 283 ppm, alterations in BUN, AST and potassium and fluid or red fluid in the lungs were observed in some animals. Two additional groups of animals were then added for 4 consecutive exposures at lower levels (55 and 156 ppm). There were no treatment-related findings observed at 55 ppm. At 156 ppm, respiratory effects and decreased activity were reported during exposure and post-exposure, irregular gait was observed.

These studies demonstrated that in addition to a relatively steep dose-response curve for inhalation toxicity to methyl bromide, cumulative toxicity following repeated inhalation exposures is also significant. Based on the results of this study, the study author selected 156 ppm as a MTD for repeated 7-hr exposures, but noted that selection of dose levels for a long-term inhalation toxicity study on methyl bromide in the dog is difficult due to cumulative toxicity. The dog 4-week inhalation toxicity study on methyl bromide (MRID 43386802) is reviewed separately (this HED Doc. No.) and further demonstrates the cumulative effects of repeated exposures.

This study is classified acceptable/nonguideline. The deficiencies outlined below are not considered to significantly affect the conclusions of the study.

B. Study deficiencies: Technical problems caused variations from target concentrations at some exposure levels. The cause of these variations was not discussed in the report. A small number of animals were tested in each group. No control animals were included. However, this was a range-finding study for longer-term exposure studies, and was not required by the U.S. EPA. A new study is therefore not required.

014497

[Methyl bromide, tech.]

Subchronic Inhalation Study (82-4)

EPA Reviewer: Linnea J. Hansen, Ph.D.
Toxicology Branch (7509C)
EPA Secondary Reviewer: Byong-Han Chin, Ph.D.
Reregistration Branch I (7509C)

Linnea J. Hansen Date 1/29/01

Byong-Han Chin Date 1/31/01

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (4-week) Inhalation Toxicity - Dog; OPPTS 870.3465 [§82-4] (submitted under §81-3)

DP BARCODE: 210630
P.C. CODE: 053201

SUBMISSION CODE: S479359
TOX. CHEM. NO.: 555

TEST MATERIAL (PURITY): Methyl bromide, tech. (100% a.i.)

SYNONYMS: *Bromomethane*

CITATION: Newton, Paul E. (1994) A Four Week Inhalation Toxicity Study of Methyl Bromide in the Dog. Pharmaco LSR, Inc. Toxicology Services North America (East Millstone, NJ). Laboratory Study No. 93-6068 (Sponsor Study No. MBIP-33.0-DOGRF-PHARM), September 14, 1994. MRID 43386802. Unpublished.

SPONSOR: Chemical Manufacturers Association (Methyl Bromide Industry Panel), Washington, D.C.

EXECUTIVE SUMMARY: In a subchronic inhalation study (MRID 43386802) methyl bromide (tech., 100% a.i.) was administered 7 hours/day, 5 days/week to 4 beagle dogs/sex/dose by whole body exposure at target concentrations of 0, 5, 10/150, 25, 50 or 100 ppm (actual mean concentrations 0, 5.3, 11.0/158.0, 26.0, 53.1 or 102.7 ppm; equivalent to 0, 0.021, 0.043/0.614, 0.101, 0.206 or 0.399 mg/L), as follows: "interim" sacrifice - 2 dogs/sex, 0 ppm group and all dogs, 25, 50 and 100 ppm groups, for almost 5 weeks (total 24 exposures); "terminal sacrifice" - 2 dogs/sex, 0 ppm group and all dogs, 5 ppm group for almost 7 weeks (total 34 exposures); and all dogs, 10/150 ppm group for almost 5 weeks at 10 ppm (24 exposures), then at 150 ppm for 6 additional exposures (also "terminal" sacrifice). In addition to standard evaluations performed in a guideline subchronic study, a neurological examination was performed by a veterinarian after termination of exposures and serum bromide levels were measured weekly.

"Interim" sacrifice: At 53 ppm, decreased activity was observed in 2/8 dogs during exposure beginning on day 14. At 103 ppm, decreased activity (starting on day 9 in 3 dogs; eventually in all), tremors (1 male, 10th exposure) and decreased body weight in both sexes (-9% less than controls due to cumulative weight loss of -0.6 kg, males and -1.0 kg, females) were observed. Serum bromide ion concentration was increased for both sexes at 53 and 103 ppm (+160% to +500%), with only slight increases at 26 ppm (by day 19, +44%, males and +37%, females). No neurological deficits were observed during the post-exposure neurological examination. There were no treatment-related effects on food consumption, ophthalmological findings, hematology

parameters, organ weights or gross findings at any dose, and no effects observed at ≤26 ppm. **The systemic toxicity LOAEL for 5 weeks (24 exposures) is 53 ppm (0.206 mg/L), based on decreased activity. The NOAEL is 26 ppm (0.101 mg/L).**

"Terminal" sacrifice: At 5 ppm after 34 exposures, 2/8 dogs (both females) showed unresponsiveness and/or depressed appearance at the neurological exam. At 158 ppm (increased from 10 ppm after the 24th exposure), 3 males were sacrificed after 6 exposures due to opisthotonos, paddling gait of all limbs, opening/closing of jaws and convulsions. Decreased activity (after 2 exposures); poor condition and in 1 male, tremors and prostration (6 exposures); at 2 days after the last exposure, ataxia, intention tremor, nystagmus, marked depression and inability to perform postural responses; decreased body weight/weight loss (males -24%/loss of 1.2 kg; females loss of 0.7 kg); increased urinary bilirubin and protein; vacuolization of the cerebellar granular layer (8/8); olfactory epithelial degeneration in the nasoturbinal tissues (8/8) and intracytoplasmic vacuolization of the adrenal gland *zona fasciculata* (4/4 males) were observed. Serum bromide levels increased by up to ~400% in both sexes. No treatment-related findings were reported in animals exposed to 11 ppm for 5 weeks. There were no treatment-related effects on food consumption, ophthalmological findings, hematology parameters, organ weights or gross findings in this study at any dose. **The systemic LOAEL (threshold) for a 7 weeks (34 exposures) is 5 ppm (0.021 mg/L), based on decreased responsiveness in females. The NOAEL (threshold) is <5 ppm.**

This subchronic toxicity study is classified **Acceptable/Non-Guideline** (§82-4). A subchronic inhalation study in the dog was not required by the US EPA for reregistration of methyl bromide; this study was conducted as a range-finding study for a chronic inhalation study in dogs to satisfy data requirements of California Department of Pesticide Regulation.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Methyl bromide
   Description: technical grade colorless gas
   Lot/Batch #: SLV (Great Lakes Chemical Corp.)
   Purity: 100% a.i.
   Stability of compound: not described in this report. However, methyl bromide has previously been reported to be stable stored in compressed gas cylinders
   CAS #: 74-83-9

2. Vehicle and/or positive control: None
3. Test animals: Species: dog

Strain: Beagle
Age and weight at study initiation: 6-7 months. Males 9.5-12.5 kg; females 8.1-10.3 kg.
Source: Marshall Farms, U.S.A., Inc., North Rose, NY
Housing: individual housing in elevated metal grid cages during non-exposure periods; in stainless steel cages during exposures
Diet: Purina Certified Canine Diet #5007, 400 g provided each night.
Water: tap water, provided ad libitum
Environmental conditions:
Temperature: 68 to 73°F; Humidity: 32 to 70%
Air changes: not indicated; Photoperiod: 12 hr light/12 hr dark
Acclimation period: at least 14 days

B. STUDY DESIGN:

1. In life dates - start of exposures: April 18, 1994;
end (last day of terminal sacrifice): June 3, 1994.
2. Animal assignment
Animals were randomly assigned to the groups in Table 1 by a method designed to equalize mean body weights.

TABLE 1: STUDY DESIGN[1]

Group No.; Target Exposure Level, ppm	No. Animals Assigned to Each Group													
	Initial		Clinical lab evaluation				Necropsy				Microscopic Pathology			
			Interim		Terminal		Interim		Terminal		Interim		Terminal	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
(I) 0	4	4	2	2	2	2	2	2	2	2	2	2	2	2
(II) 5	4	4	--	--	4	4	--	--	4	4	--	--	4	4
(III) 10/150[2]	4	4	--	--	4	4	--	--	4	4	--	--	4	4
(IV) 25	4	4	4	4	--	--	4	4	--	--	4	4	--	--
(V) 50	4	4	4	4	--	--	4	4	--	--	4	4	--	--
(VI) 100	4	4	4	4	--	--	4	4	--	--	4	4	--	--

1 Table copied from Page 16 of MRID 43386802.
2 Exposure was increased from 10 ppm to 150 ppm beginning on the last day of exposure week 5 (study day 35).

Rationale for dose selection: The exposure levels used in this study were based on the results of a previous range-finding study in which beagle dogs were exposed for up to 4 days (MRID 43386801; see review, this HED Doc. No.).

This study was initially designed to provide a 4-week exposure for each group. However, actual exposure periods were extended to about 5 weeks for half of group I and all of groups IV through VI. The remainder of group I and all 4 animals in groups II and III were continued on the study for an exposure period of 6 to 7 weeks to assess cumulative toxicity of methyl bromide. The sacrifice at 5 weeks was termed the "interim sacrifice" and the 6 to 7-week sacrifice, the "terminal sacrifice". Group III exposures were increased to 150 ppm for 6 additional exposure days following exposure to 10 ppm for 5 weeks. All groups were exposed for 7 hrs/day, 5 days/week. Exposure start/stop dates and date of sacrifice are as follows:

Group No.- ppm	Exposure start	Exposure stop	No. exposure days	Date of Sacrifice
I - 0 ppm	April 18	May 19	24	May 19 or 20
I - 0 ppm	April 18	June 2	34	June 3
II - 5 ppm	April 18	June 2	34	June 3
III - 10 ppm	April 18	May 19	24	May 28 or 31
III - 150 ppm[1]	May 20	May 27	6	May 28 or 31
IV - 25 ppm	April 18	May 19	24	May 19 or 20
V - 50 ppm	April 18	May 19	24	May 19 or 20
VI - 100 ppm	April 18	May 19	24	May 19 or 20

1 all animals in this group were exposed to 10 ppm for 24 exposures prior to 150 ppm.

2. **Generation of the test atmosphere and description of the chamber:** Prior to study start, tests to optimize equipment and operating conditions were conducted to ensure that stable test atmospheres were generated at each exposure level. Animals were exposed in whole-body 6,000 liter exposure chambers (Harford Glass, Aberdeen, MD), operated dynamically under slight negative pressure. Airflow rates ranged from 1,212 to 1,317 lpm, with air change times ranging from 4.6-5.0 minutes. These conditions were stated to be sufficient for maintaining the animal loading factor below 5% and chamber oxygen above 19%.

Test material from a compressed gas cylinder was mixed with chamber air in a gas bag prior to entering the exposure chamber. This mixture was pumped through a flowmeter and metering valve and then to the exposure inlet portal. Control animals were exposed to air only. At each exposure, animals were rotated so that their placement within the chamber varied each time to provide uniform exposure. All animals remained in the chambers for 30 minutes after exposure termination to allow clearance of test material. Chambers were exhausted through a coarse filter, a HEPA filter and charcoal.

Chamber conditions: Ambient conditions within the test chambers were evaluated every 30 minutes during exposures. Time to equilibrium (T_{99}) for all exposure levels was 21-23 minutes. Chamber oxygen was measured at 21%, relative humidity ranged from 34%-67% (exposure group means 48-53%) and temperature from 19-25°C (exposure group means 21-22°C).

Analytical Chemistry: Test atmospheres were sampled hourly from the breathing zone during exposures using a Hewlett Packard Gas Chromatograph II. Analytical values were measured by comparison to calibrated response curves at the same instrument settings. The uniformity of the chamber concentrations was evaluated prior to the study by analyzing samples from several ports.

Test atmosphere concentration: Results of chamber atmosphere analyses are summarized in Table 1,

below. With the exception of a few average daily samples at each exposure level during the study that varied by more than 15% from the target concentration, most daily values were within acceptable range of target concentration. The mean analytical concentrations were all within 10% of the target. The variations that did occur are not anticipated to significantly alter the conclusions of the study.

TABLE 2: ANALYTICAL DATA FROM EXPOSURE CHAMBER SAMPLING[1]

Test group	Target Conc. (ppm)	Mean Analytical Conc. (ppm)	Range of Analytical Conc. (ppm)	MMAD μm
Control	0	0.0	0.0	nd[2]
Low (LCT)	5	5.3±0.4	4.7-6.4	nd
Mid 1 (MCT1)	10 150[2]	11.0±0.58 158±7.0	10.0-12.4 142.8-166.2	nd
Mid 2 (MCT2)	25	26.0±1.3	24.0-28.7	nd
Mid 3 (MCT 3)	50	53.1±4.2	42.5-59.9	nd
High (HCT)	100	102.7±9.2	75.3-112.1	nd

1 Data extracted from page 33 and from Tables B-2 through B-10 (Appendix B) of MRID 43386802.
2 All animals from the 10 ppm group were exposed to 150 ppm for 6 days after exposure at 10 ppm for 24 days.
Nd No data provided in the study report.

Particle size determination - There was no indication in this report that particle size was evaluated. However, in the "up and down" inhalation study in the dog (MRID 43386801) conducted by this laboratory prior to this study, no aerosol was measured.

3. Statistics - Body weight/cumulative weight change, food consumption data were analyzed through week 5. Pretest clinical laboratory studies were also analyzed. No statistical analyses were performed on treated groups with ≤2 animals or when the standard deviation for controls or more than one group was 0 due to lack of variance. Equality of variance was first evaluated using Bartlett's test. Where variances were equal, parametric one-way ANOVA with the F distribution was used, followed by Dunnett's test when significant differences among group means were observed. Parametric data were also analyzed for trend by standard regression with a test for trend and lack of fit. Where variances were not equal, Kruskal-Wallis test was used, followed by Dunn's Rank Sum test where significant differences among means were observed. Nonparametric data were also analyzed for trend by Jonckheere's test for monotonic trend. Statistical significance was identified at 1% for Bartlett's test and 5% and 1% for all other tests (two-sided risk level).

C. METHODS:

1. Observations:

Animals were inspected twice daily (pre- and post-exposure) for gross findings and mortality. A daily observation for clinical signs of toxicity was conducted once during each exposure. Detailed clinical examinations of each animal were conducted pretest, then weekly. In addition, a neurological examination was conducted by a veterinarian pretest, at Week 4 and at termination.

2. Body weight

Animals were weighed twice pretest, weekly during exposure and at termination (after fasting).

3. Food consumption

Food consumption (g/kg body weight/day) was determined pretest and weekly thereafter (amount recorded 4X/week). Food efficiency was not determined.

4. Ophthalmoscopic examination

An ophthalmoscopic evaluation was performed prior to exposure (April 14, 1994), at week 4 (May 16, 1994) and prior to terminal sacrifice (June 2, 1994).

5. Blood was collected from all animals (fasted overnight) for hematology and clinical analysis at pretest and interim sacrifice or terminal sacrifice. Samples were taken by jugular venipuncture with no anesthesia. In addition, serum bromide levels were measured weekly for all dogs. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Erythrocyte morphology
X	(Partial thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
X	Bromide	X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophoresis
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected for 16 hrs from water-deprived animals prior to exposure, at the interim sacrifice for animals assigned to that group and at terminal sacrifice for the remaining animals. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
X	Volume (16-Hr)	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.
X	Esophagus*	X	Bone marrow*		nerve*
X	Stomach*	X	Lymph nodes*	X	Spinal cord (3 levels)[T]
X	Duodenum*	XX	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)[T]
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+	X	Adrenal gland*
X	Rectum*	X	Urinary bladder*	X	Lacrimal gland[T]
XX	Liver*+	XX	Testes*+	X	Mammary gland[T]
X	Gall bladder*	XX	Epididymides	XX	Parathyroids*++
X	Pancreas*	X	Prostate	XX	Thyroids*++
			Seminal vesicle		
	RESPIRATORY	XX	Ovaries		OTHER
X	Trachea*	X	Uterus*	X	Bone
XX	Lung*			X	Skeletal muscle
X	Nose			X	Skin
X	Pharynx			X	All gross lesions and masses*
X	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines.
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ.
++ Organ weight required for non-rodent studies.

For examination of nasopharyngeal, tissues, 4 sections were cut from the intact, formalin-preserved skulls of each animal: (1) between the upper incisor tooth and incisive papilla; (2) between the incisive papilla and the first palatal ridge; (3) between the second palatal ridge and fist upper molar and (4) between the first upper molar and the nasopharynx, including the nasopharynx. The larynx was examined in 2 slides cut through (1) the ventral diverticulum and (2) the ventral seromucous glands at the base of the epiglottis.

II. RESULTS

A. Observations

1. Toxicity - During exposure: No clinical signs of toxicity were observed at ≤26 ppm. At 53 ppm, decreased activity was first observed in 2 dogs on day 14. Thereafter, 1 to 4 of the dogs in that group showed decreased activity (lack of interest when approached) on most exposure days. At 103 ppm, 3 dogs showed decreased activity on day 9; by day 12, all animals had decreased activity during most of the remainder of the exposure period. One animal (sex not indicated) had tremors on day 10. When exposure of Group III animals (11 ppm) was increased to 158 ppm on exposure day 25, decreased activity was observed in all animals beginning on day 27. All were in poor condition by day 30, including one male that was prostrate and had tremors.

Post-exposure weekly detailed examinations: One male at 26 ppm and 1 at 53 ppm showed excessive salivation after 3 and 4 weeks' exposure, respectively. TB-I agreed with the study author, who did not consider this treatment-related because it was not observed at higher exposure levels nor in females. Irregular gait was reported at 158 ppm (study Week 6) in all females and 1 male and was considered treatment-related.

Clinical veterinarian examination of moribund animals, post-exposure: On the sixth exposure day of the Group III animals to 158 ppm (5/28/94), severe signs of toxicity were observed in 3 males, which were then humanely sacrificed. These included opisthotonos, paddling gait (all limbs), opening/closing of jaws and convulsions. Exposure was terminated for the remaining 4 females and 1 male on 5/31/94.

Neurological examination: No neurologic deficits were identified in any dose group at Week 4. The remaining animals continued in the study (0, 5 and 11/158 ppm) were reexamined following termination of exposures. Although no postural or reflex deficits were reported, after 6 weeks' exposure, two females in the 5 ppm group (2575F, 2577F) were unresponsive and motionless: one of these appeared depressed and lifted the left forelimb. The neurologist considered the toxicological significance of these behaviors to be unclear and the study authors did not consider them treatment-related. However, because decreased activity or depression was observed in animals exposed to higher doses for fewer exposures (and toxicity from methyl bromide exposure appears to be cumulative), TB considered the decrease in activity to be a treatment-related threshold response.

In the dogs exposed to 158 ppm after 5 weeks' exposure to 11 ppm, all animals showed significant clinical signs when examined 2 days post-exposure. Ataxia, base-wide stance, intention tremor and nystagmus were observed in all dogs. Two dogs also showed marked depression and inability or unwillingness to stand and perform postural responses. The dominant symptoms observed were considered by the neurologist to be consistent with cerebellar/vestibular dysfunction and with the known effects of methyl bromide on the central nervous system.

No individual animal clinical finding data for the in-chamber observations during exposure were provided in this study. It therefore could not be determined whether the animals that showed clinical signs during exposure were the same ones showing signs post-exposure or during the special neurological examination.

2. Mortality - No animals were found dead, but 3 males exposed to 158 ppm for 6 days were sacrificed due to severe toxicity (convulsions, opening/closing of jaws, opisthotonos and paddling gait of fore- and hindlimbs).

B. Body weight and weight gain - Mean body weights and cumulative weight gain are shown below in Table 3:

TABLE 3: MEAN BODY WEIGHT AND CUMULATIVE WEIGHT GAIN VALUES (KG)[1]

	EXPOSURE LEVEL IN PPM					
Sex/Week of exposure	0	5	11/158[2]	26	53	103
Males Pretest	11.3	11.2	11.0	11.4	11.1	10.9
Week 1	11.1	11.0	10.8	11.1	10.8	10.4
Week 2	11.2	11.3	11.1	11.4	11.0	10.4
Week 3	11.4	11.2	11.1	11.5	10.9	10.2
Week 4	11.3	11.4	11.2	11.6	11.3	10.3
Week 5	11.3	11.3	10.9	11.8	11.3	10.3
Week 6	11.1[3]	11.6	8.3[4]	--	--	--
Week 7	11.5[3]	11.6	–[5]	--	--	--
Cumulative weight gain	0.75 (0.1, week 5)	0.4	0.2/-1.2	0.5	0.1	-0.6*
Females Pretest	8.9	9.2	9.3	9.1	9.1	9.1
Week 1	8.7	8.7	9.2	8.8	8.5	8.6
Week 2	8.8	8.9	9.4	8.9	8.6	8.5
Week 3	8.9	8.9	9.5	9.0	8.6	8.3
Week 4	9.0	9.0	9.7	9.2	8.8	8.4
Week 5	9.0	8.7	9.5	9.2	8.9	8.2
Week 6	8.8[3]	9.2	8.6	--	--	--
Week 7	8.7[3]	9.1	8.6	--	--	--
Cumulative weight gain	0.3 (0.1, week 5)	0.0	0.3/-0.7	0.1	-0.2	-1.0*

1 Data extracted from Tables F-2, F-3, F-8 and F-9 (Appendix F), MRID 43386802. All values calculated by study author. Data analyzed statistically through Week 5 only. N = 4 except where noted.
2 Exposures were increased from 11 ppm to 158 ppm during weeks 5 and 6; gain values indicates cumulative gain through week 4 (11 ppm exposures only)/ week 6 (11 and 158 ppm).
3 N = 2 (half of control animals terminated at the interim sacrifice).
4 N = 1 due to humane sacrifice of 3 males.
5 -- not determined (exposures terminated).
* Statistically significant, $p \leq 0.05$.

No significant body weight or weight gain changes related to treatment were reported in animals exposed at ≤53 ppm. At 103 ppm, mean body weight was decreased in males and females by 9% compared to controls at Week 5 (end of exposure). Both sexes at 103 ppm showed weight loss (statistically significant) compared to controls (at Week 5, -0.6 kg, males and -1.0 kg, females vs. 0.1 kg, controls for both males and females).

At 11/153 ppm during weeks 5 and 6, mean body weight of males was -24% less than controls and a weight loss of -1.2 kg, vs. 0.2 kg gain in controls, was observed by Week 6. In females of this group, mean body weights were comparable to controls at termination (8.6 kg vs. 8.7 kg, controls) but a weight loss of -0.7 kg at termination, vs. 0.3 kg gain in controls, was observed due to higher mean pretest weights of the Group III females relative to controls. Mean body weight at week 6 was -8% below pretest weight.

C. Food consumption - No significant differences in food consumption were reported in animals exposed up to 103 ppm. However, after exposure of the Group III animals was increased to 158 ppm at the end of Week 5, food consumption (g/kg/day) in both males and females decreased by 47% and 35%, respectively, during week 6. This decrease was considered treatment-related and corresponded with decreased body weight gain.

D. Ophthalmoscopic examination - No treatment-related abnormalities were reported.

E. Blood work

1. Hematology - Hematology findings pretest and at termination are shown below in Table 4:

TABLE 4: SELECTED HEMATOLOGICAL PARAMETERS, TERMINAL SACRIFICE[1]

Sex/Parameter (Units)	Exposure level in ppm					
	0	5	11/158	0	5	11/158
	Pretest			Termination		
Males	(N = 4)	(N = 4)	(N = 4)	(N = 2)	(N = 4)	(N = 1)
Hgb (g/dl)	15.4	14.6	15.9	15.8	14.6	16.4
Hct (%)	46.5	43.6	47.6	46.9	42.9	48.3
RBC (mil/ul)	6.77	6.45	7.06	6.95	6.63	7.17
Retic (thous/ul)	1.4	1.4	1.4	2.4	2.0	0.4
Females	(N = 4)	(N = 4)	(N = 4)	(N = 2)	(N = 4)	(N = 4)
Hgb (g/dl)	16.5	15.4	15.1	16.4	14.6	13.9 (-15)
Hct (%)	50.7	46.2	45.7	48.2	42.7	41.4 (-14)
RBC (mil/ul)	7.34	6.82	6.55	6.87	6.38	6.05 (-12)
Retic (thous/ul)	1.8	1.6	1.6	1.6	2.0	0.2 (-88)

1 Data extracted from Tables H-2, H-3, H-6 and H-7 (Appendix H), MRID 43386802.
2 Numbers in parentheses indicate percent decrease relative to control values.

There were no treatment-related effects on hematology parameters in animals exposed up to 103 ppm or in males at any exposure level. At termination in females exposed to 11/158 ppm, HGB, HCT and RBC were decreased by 12-15% relative to controls but only by 7-8% relative to same-treatment group pretest values. The study author did not consider these decreases to be biologically significant due to their relatively

small magnitude. TB considers these effects to be of unclear significance but they may represent a marginal effect on red blood cells. Increased urinary bilirubin was also found, although males rather than females showed greater effects and individual animal data did not show a strong correlation. The decrease in reticulocyte count is not considered biologically significant, since similar low values were found all groups of the interim sacrifice animals of both sexes.

2. Clinical chemistry - Selected clinical chemistry parameters showing statistically significant differences from controls at the interim measurement are shown below in Table 5:

TABLE 5: SELECTED CLINICAL CHEMISTRY VALUES, INTERIM SACRIFICE[1]

	Exposure level in ppm			
Sex/Parameter (Units)	0	26	53	103
Males	(N = 2)	(N = 4)	(N = 4)	(N = 4)
CK (IU/L)	279	248	206	130* (-53%)[2]
Chloride (mEq/L)	109	110	113	115* (+5.5%)
Females	(N = 2)	(N = 4)	(N = 4)	(N = 4)
CK (IU/L)	611[3]	226 (-63)	154 (-75)	93 (-85)
Chloride (mEq/L)	107	109	113	115 (+7.4%)

1 Data extracted from Tables J-22 through -25, MRID 43386802.
2 Numbers in parentheses indicate percent decrease or increase relative to control values.
3 Mean of two females, 249 and 972 IU/L.

In males at 103 ppm, a statistically significant decrease in creatine kinase was observed (-53% less than controls). Decreases were also observed in females at all dose levels (-63 to -85%; not significant). One of the control females showed an abnormally high CK level (see Table 4 footnotes): if the 103 ppm females were compared to the other female, a decrease of -63% was observed. TB agreed with the study author that it was not biologically significant since increases, rather than decreases, are usually considered toxicologically meaningful and the activity of this enzyme is normally variable. Chloride ion concentration was also slightly increased in males (+5.5%; statistically significant) and females (+7.4%; not significant). Although this increase appeared to be treatment-related, it was probably not biologically significant. There were no treatment-related differences identified at the terminal sampling time in Groups I (5 ppm) or III (11/158 ppm), but only 1 male from Group III was available at the time of sampling and therefore the data for males are inadequate to assess effects at 158 ppm.

3. Serum bromide concentrations

Dose-related increases in serum bromide levels were observed in both sexes. Mean concentrations of serum bromide at selected time points are shown below in Table 6:

TABLE 6: SELECTED MEAN SERUM BROMIDE CONCENTRATIONS (μg/ml)DURING STUDY[1]

Sex/study day (Date)		Exposure levels in ppm					
		0	5	11/158[2]	26	53	103
Males	-4 (4/14)	62	67	65	72	76	62
	5 (4/22)	93	79	83	94	126	194
	19 (5/6)	80	79	92	115	218	373
	26 (5/13)	75	76	81	115	204	403
	33 (5/20)	81[4]	83	**130**[3]	-[2]	-	-
	40 (5/27)	81[4]	92	**380**	-	-	-
	43 (5/30)	83[4]	83	**430**	-	-	-
	47 (6/3)	69[4]	76	-	-	-	-
Females	-4 (4/14)	72	62	59	59	62	63
	5 (4/22)	82	77	74	91	129	200
	19 (5/6)	83	82	83	114	199	393
	26 (5/13)	73	73	82	107	190	435
	33 (5/20)	72[4]	76	**106**	-	-	-
	40 (5/27)	85[4]	87	**388**	-	-	-
	43 (5/30)	82[4]	81	**266**	-	-	-
	47 (6/3)	67[4]	73	-	-	-	-

1 Data extracted from Tables N-2 through N-15 (Appendix N) of MRID 43386802. Values are means calculated by TB-I from individual animal data and are not analyzed statistically.

2 No measurements taken due to termination of exposure group.

3 Boldface indicates measurements taken after exposure levels were increased to 158 ppm. For males, N = 1.

4 N = 2 due to removal of half of the control animals of each sex for the interim sacrifice.

At 5 ppm and 11 ppm, mean serum bromide levels were similar to controls/pretest levels. At 26 ppm, only slight increases were reported at study day 12-19 (+44% and +37%, males and females at day 19). At 53 and 103 ppm, increases were marked and dose-related (+160% to +500% above concurrent controls). Levels increased during the first weeks of exposure, but appeared to reach equilibrium within each dose group by the end of the exposure periods. When exposure to the 11 ppm animals was increased to 158 ppm, a sharp increase in serum bromide was observed (up to about +400% above concurrent controls). The study author noted that there did not appear to be a relationship between the neurological symptoms observed in this study and the serum bromide levels, since animals at 103 and 158 ppm had similar bromide levels but the 158 ppm animals showed more severe signs of toxicity.

F. Urinalysis - Individual animal values only were provided. There were no treatment-related findings on any of the parameters at the interim sacrifice (all animals evaluated). However, treatment-related findings were reported in animals following exposure to 158 ppm relative to

the other groups at terminal sacrifice. Selected urinalysis parameters from terminal sacrifice animals are shown below in Table 7. Data are presented for each individual animal:

TABLE 7: SELECTED INDIVIDUAL URINALYSIS FINDINGS AT TERMINAL SACRIFICE[1]

Sex/Parameter	Exposure levels in ppm		
	0	5	11/158[2]
Males	(N = 2)[3]	(N = 4)	(N = 4)
Bilirubin[4]	Small/Neg Neg	Small Small Neg Neg	Large/Pos Large/Pos Large/Pos Large/Pos
Protein (mg/dl)	30 Neg	30 30 30 Trace	30 ≥300/2+ ≥300/2+ ≥300/2+
Females	(N = 2)	(N = 4)	(N = 4)
Bilirubin[4]	Neg Neg	Neg Small Neg Neg	Small Small Small Moderate/Pos
Protein (mg/dl)	Trace Neg	Neg 100/Tr 30 Neg	Trace 30 ≥300/+3 ≥300/+2

1 Data extracted from Tables K-10 and K-11, MRID 43386802. Not analyzed statistically.
2 Exposure concentrations were increased from 11 ppm to 158 ppm for the final 6 exposures.
3 Two control animals were sacrificed at the interim sampling at Week 4 (May 14, 1994).
4 In addition to a visual determination of bilirubin in the urine by unusual color, Ictotest® was used to confirm the presence of bilirubin (small, moderate or large when positive).

Bilirubin was increased in all males and in 1 female at 158 ppm. Protein was increased in 3 males and 2 females. These effects are considered treatment-related.

G. Sacrifice and pathology

1. Organ weight - No biologically significant treatment-related effects on absolute or relative organ weight were reported. Decreased spleen weights (-55%) were observed in females at 5 ppm/34 exposures relative to controls, but these were considered sporadic.

2. Gross pathology - No treatment-related gross findings were reported.

3. Microscopic pathology

a) Non-neoplastic - Several microscopic lesions appeared to be treatment-related and are summarized below in Table 8:

TABLE 8: SELECTED NON-NEOPLASTIC MICROSCOPIC LESIONS, ALL ANIMALS[1]

	Exposure level in ppm					
Sex/organ/lesion	0	5	11/158[2]	26	53	103
Males (N = 4, all groups)						
Adrenal gland, intracytoplasmic vacuoles, *zona fasciculata* -						
minimal	0	0	1	0	0	0
slight	0	0	1	0	0	0
moderate	0	0	2	0	0	0
Total	0	0	4	0	0	0
Brain, vacuoles, granular layer of cerebellum - minimal	1	0	4	0	0	1
Nasoturbinal tissues[2], degeneration of olfactory epithelium -						
moderate	0	0	1	0	0	0
severe	0	0	3	0	0	0
Total	0	0	4	0	0	0
Females (N = 4, all groups)						
Adrenal gland, intracytoplasmic vacuoles, *zona fasciculata* -						
minimal	0	0	0	0	1	0
slight	0	0	1	0	0	0
moderate	0	0	0	0	0	0
Total	0	0	1	0	1	0
Brain, vacuoles, granular layer of cerebellum - minimal	0	0	4	0	0	0
Nasoturbinal tissues[2], degeneration of olfactory epithelium -						
moderate	0	0	1	0	0	0
severe	0	0	3	0	0	0
Total	0	0	4	0	0	0

1 Data extracted from Tables IIA-C, Appendix 0 (Pathology Report) of MRID 44386802.

2 Values shown represent total combined incidence from the nasoturbinal tissue sections. A total of 4 sections were taken (see Methods for description). Lesions were observed in Sections 3 and 4.

Microscopic lesions were observed in the brain, olfactory epithelium and adrenal gland of Group III animals (11 ppm increased to 158 ppm). Minimal vacuolization of the granular layer of the cerebellum was observed in all males and females in Group III. This finding was also observed in 1 male each at 0 and 103 ppm interim sacrifice but was not considered treatment-related in those groups because a dose-

response was not observed. Degeneration of olfactory epithelium (moderate to severe) in the nasoturbinal tissues was observed in all animals exposed to 158 ppm. Intracytoplasmic vacuolization of the adrenal gland *zona fasciculata* was also observed in all males (minimal to moderate) and 1 female (slight). The adrenal gland effects may have been stress-related. Because the microscopic effects observed in the Group III animals were not observed at ≤103 ppm, they are attributed to the 158 ppm exposure, although exposure to 11 ppm may have shortened the latency for appearance of these effects.

b) Neoplastic - There were no treatment-related increases in neoplastic lesions.

III. DISCUSSION

A. TB-I agreed with most of the conclusions of the study author, but considered the mild clinical signs of toxicity in the lowest dose group tested (5 ppm) to be treatment-related for the reasons described below.

This repeated-exposure inhalation study in the beagle dog was conducted to establish appropriate chamber concentrations for a long-term inhalation study. It tested at exposure levels of 0, 5, 11 (later increased to 158), 26, 53 or 103 ppm for different exposure periods. Although originally intended to be a 4 week study for all groups, during the study the study design was modified so that some animals were exposed for 5 weeks (0, 11, 26, 53 and 103 ppm), some for 7 weeks (0 and 5 ppm) and the 11 ppm group received additional exposures (*up to 6*) *at 158 ppm after the initial 24 exposures at 11 ppm.*

Dogs exposed to methyl bromide for a total of 24 exposures showed signs of toxicity at chamber concentrations of 53 ppm and 103 ppm. No toxicity was reported ≤26 ppm. At 53 ppm, only clinical signs of toxicity (decreased activity during the 14th exposure) were observed in 2/8 animals. At 103 ppm, all animals showed decreased activity; weight loss also occurred. The decrease in activity seen in animals at 53 ppm or above during the exposure time is difficult to judge whether or not it is due to treatment without body temperature data. Under the circumstances we can call it conservatively as a treatment related finding. Other changes at 103 ppm included decreased serum creatine kinase and increased chloride ion; however, these effects were not considered biologically significant.

Dogs exposed to 11 ppm for a total of 24 exposures showed no signs of toxicity. However, when exposures of this group were increased to 158 ppm, pronounced clinical signs of neurotoxicity were reported within 1-2 exposures. Three males were humanely sacrificed after 6 exposures due to opisthotonos, paddling gait of all limbs, opening/closing of jaws and convulsions. In the surviving animals, ataxia, base-wide stance, intention tremor, nystagmus, depression and inability to perform postural responses in the neurological examination. Neuropathological effects (minimal vacuolization of the granular layer of the cerebellum) were also observed in all animals. The neurobehavioral effects are consistent

with the pathology observed in the cerebellum. In addition, olfactory epithelial degeneration of the nasoturbinal tissues was reported in all animals (moderate to severe). Minimal increases in intracytoplasmic vacuolization of the adrenal gland *zona fasciculata* in males also appeared to be treatment-related. Increases in urinary bilirubin in males and protein in both sexes were considered treatment-related. A slight decrease in red blood cell parameters in females may have been a marginal treatment-related effect. The extent to which prior exposures to 11 ppm contributed to the severe effects observed at 158 ppm cannot be determined, but some cumulative toxicity probably resulted.

Of the 8 dogs continued on the study at 5 ppm for a total of 34 exposures, 2 females showed unresponsive behavior and/or depressed appearance/decreased responsiveness at the neurologic examination following the last exposure, although no postural or reflex deficits were reported. The study authors did not consider this finding to be treatment-related. However, TB-I considered them to be possibly treatment-related and representing a threshold effect level because (1) 2 animals were affected and (2) cumulative toxicity was clearly observed at higher exposure levels with repeated exposures and (3) similar effects of decreased activity were observed at higher exposure levels of shorter duration.

In addition to dose-dependence, toxicity of methyl bromide appeared to show a strong cumulative effect with increasing numbers of exposures. This can be observed when considering the findings of this study together with the acute range-finding study (MRID 43386801; see review for details, this HED Document). Based on the results of the 4-day exposure study, an MTD of 170 ppm was selected; however, in this study, exposures of 158 ppm proved excessive after only 6 days. Furthermore, no clinical signs were reported in animals exposed at 56 ppm for 4 days in the range-finding study, but by day 14, decreased activity was reported at 53 ppm in this study. The study author concluded that selection of exposure levels for a long-term (1 year) inhalation study would be difficult due to the cumulative toxicity of methyl bromide.

Serum bromide levels were also analyzed throughout this study. Levels were increased at 53 and 103 ppm, but only marginally at 26 ppm. When exposure of the 11 ppm animals was increased to 158 ppm, levels sharply increased. The toxicological significance of this increase is unclear. TB-I agreed with the study authors that the relationship of serum bromide levels to toxicity was uncertain, since the levels of the 103 ppm group were comparable or higher than the 158 ppm group, but the latter group showed more severe effects.

B. Study deficiencies - The study report did not indicate whether particle size was evaluated during this study. In the companion acute up and down and 4-day study report (MRID 43386801; see review, this document no.), it was stated that particle size was analyzed hourly during exposures using a TSI Particle Sizer and that no detectable aerosol was observed. Since this study was conducted using the same exposure equipment and exposures were generated by essentially the same procedures, it is unlikely that particle

sizes were unacceptably large. However, TB-I requests that if any particle size analyses were conducted during this study, they should be submitted as confirmatory data. (2) Individual animal clinical observation data for observations made during exposures were not provided so that correlation with animals showing post-exposure signs of toxicity could not be made. (3) Blood creatinine was not evaluated in the clinical chemistry profile. These findings are not considered to affect the conclusions.

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Chemical:	**Methyl bromide**
PC Code:	**053201**
HED File Code	**13000 Tox Reviews**
Memo Date:	**02/14/2001**
File ID:	**TX014497**
Accession Number:	**412-02-0006**

HED Records Reference Center
12/04/2001